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Detection of additional abnormalities or co-morbidities in women with suspected intrahepatic cholestasis of pregnancy

Short title: Testing in suspected intrahepatic cholestasis of pregnancy

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Trial registration: Not applicable because study was a retrospective audit.

Contributorship: LCC and JG designed the study. FCR, MM, RH, CLK, GA , OB and JW performed data collection. FCR and RH performed data analysis. FCR, CW, LCC and JG interpreted the results. The first manuscript draft was written by FCR and re-drafted and approved by all other authors.

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Abstract

Background: Current guidelines recommend viral, autoimmune, coagulation and liver ultrasound testing in intrahepatic cholestasis of pregnancy (ICP) to exclude alternative diagnoses.

Methods: Electronic health records were searched for investigations and diagnoses in women with raised bile acid (BA) concentrations (>10 µmol/L) between January 2016-December 2017 at two UK maternity units.

Results: 531 women had a raised BA concentration (median (IQR): 18 (13-32 µmol/L)) at a median gestation of 35.1 (IQR 31.8-37.0) weeks. 250/531 (47.1%) had full virology, autoimmune and ultrasound tests. 348/531 (65.5%) had coagulation performed. Positive hepatitis B and C results were previously known. No new Epstein-Barr Virus, Cytomegalovirus or hepatitis A diagnoses were made. There were 11 positive autoimmune results, but no new diagnoses. No women had unexplained prolonged prothrombin time. No ultrasound liver (n=38) or gallbladder (n=85) abnormalities were of acute clinical significance.

Conclusion: ICP investigations provided no new diagnoses that influenced clinical management during pregnancy.

Background

Intrahepatic cholestasis of pregnancy (ICP) or obstetric cholestasis is the commonest pregnancy-specific liver disease, affecting approximately 0.7% of pregnancies in multi-ethnic populations in the UK.[1] It has a multifactorial aetiology with genetic, hormonal and environmental components,[2–4] with higher incidence noted in women of Asian-Pakistani (1.5%) and Asian-Indian (1.2%) origin.[5] ICP is diagnosed by the combination of pruritus and elevated bile acids (BA) in pregnancy, in the absence of another identified cause, with resolution of clinical symptoms and biochemical abnormalities expected within weeks of delivery.

Current guidelines advocate approaching ICP as a diagnosis of exclusion. The Royal College of Obstetricians & Gynaecologists (RCOG) Obstetric Cholestasis Green-top Guideline (last updated in 2011) recommends that 'other causes of pruritus and abnormal liver function tests should be sought', suggesting viral testing for Hepatitis A, B, C, Epstein Barr Virus and cytomegalovirus, liver autoimmune testing for chronic active hepatitis and primary biliary cirrhosis, liver ultrasound and that 'a coagulation screen should be performed'.[6] The principal aim of this testing in the antenatal period is to identify alternative causes for the clinical presentation (i.e. pruritus and raised bile acid concentrations). However, the proportion of women with suspected ICP who have additional investigations and the detection rate for alternative diagnoses or co-morbidities as a result of testing in ICP is uncertain.

Methods

All tests for bile acid concentrations performed in two London hospital maternity units between 1 January 2016 – 31 December 2017 were reviewed. Women with a peak bile acid concentration above the upper limit of normal (>10 µmol/L in both units) were identified. Data review was performed to remove records of non-pregnant women and those in whom bile acid concentration was measured following stillbirth without any clinical symptoms or preceding diagnosis of ICP. Following data cleaning, hospital laboratory databases were reviewed to determine which investigations were performed during pregnancy and the results of these investigations (biochemistry, virology, autoimmune antibodies, coagulation and liver ultrasound) and clinical outcomes were extracted from electronic health record databases. In women with abnormal investigation results and those with severe early-onset disease (defined as a peak bile acid level of ≥40 µmol/L at <32 weeks' gestation), all available electronic health records including discharge summaries and clinic letters were reviewed in order to determine whether any new diagnoses were made. Data manipulation and analysis was performed in R version 3.4.3 (The R project for Statistical Computing). ANOVA and Kruskal-Wallis tests for continuous and Chi-Squared trend test for categorical variables were used to assess for differences between peak bile acid concentration groups as appropriate.

Results

The selection of women with a peak bile acid concentration of >10 µmol/L during pregnancy during the 2-year study period is shown in Figure 1. Out of 23,927 deliveries at both hospital sites, 531 (2.2%) women had raised bile acid concentrations with first raised bile acid at a median gestation age of 35.1 weeks (interquartile range (IQR) 31.8-37.0). 184 women (34.7%) had a peak bile acid concentration ≥40 µmol/L during their pregnancy (at a median gestational age of 34.3 (IQR 32.1-36.5) weeks).

Maternal demographics

Maternal demographics in women with raised bile acids during pregnancy are shown in Table 1. There were 47 (8.9%) twin pregnancies.

Delivery and fetal outcomes

Delivery and fetal outcomes in women are shown in Table 2. Women with a peak bile acid concentration of ≥40 µmol/L delivered at earlier gestations. There were high rates of induction of labour (between 38.9% and 54.6%) in all groups, with approximately 50% of women overall being induced and 19% having planned delivery by caesarean section. Overall 19% of women delivered preterm (prior to 37 weeks' gestation), of whom 25.7% went into spontaneous preterm labour. 4% of women delivered very preterm (at <34 weeks' gestation), of whom 33.3% had spontaneous onset of preterm labour. There were two stillbirths (0.3% of births) at 31 and 35 weeks' gestation, both of which occurred in women who had a peak bile acid concentration of ≥100 µmol/L during pregnancy, and with additional maternal comorbidities alongside ICP. There was one neonatal death (0.2% of births) of one baby of twins delivered at 32 weeks' gestation by elective Caesarean section for suspected twin to twin transfusion in whom the woman had a peak bile acid concentration of 42 µmol/L.

Biochemistry testing

Other biochemistry results at time of first raised bile acid are summarised in Table 3. Women who had a peak bile acid concentration ≥40 µmol/L during pregnancy presented with higher initial raised bile acids, and with higher associated concentrations of alanine transaminase. There were 15 women (2.8%) who had raised bilirubin at time of peak bile

acid, of which 7 (1.3%) cases were elevated sufficiently (>34-42 umol/L) to cause clinical jaundice.

Virology testing

A summary of virology, autoimmune and ultrasound testing and results is shown in Table 4. 380 (71.6%) women were tested for Epstein Barr Virus (EBV): of these, 343 (90.3%) women were immune (EBV Viral Capsid Antigen (VCA) IgG positive, IgM negative), 13 (3.4%) were susceptible (EBV VCA IgG and IgM negative), and 24 women (6.3%) were both EBV VCA IgG and IgM positive which can denote recent infection. In these latter cases, further testing for Epstein-Barr nuclear antigen (EBNA) was performed. Twenty three women tested positive for EBNA denoting past (minimum of 6-8 weeks previously) rather than acute infection, and one woman tested EBNA negative; however she had a low positive EBV VCA IgM result which was reported by the virology team as 'consistent but not typical of primary EBV infection', no testing for EBV viral load was performed and no clinical diagnosis was made on review of electronic health records.

Of the 395 (74.4%) women tested for cytomegalovirus (CMV), 170 (43.0%) were immune (CMV IgG positive, CMV IgM negative/not tested in two cases), 181 (45.8%) tested negative for CMV IgM (IgG testing was not performed), 37 (9.4%) were susceptible to CMV infection (CMV IgG and IgM negative), and seven women (1.7%) tested positive for CMV IgG and IgM which can denote recent infection. In these seven women, three were tested for CMV DNA and were all negative, two had repeat CMV serology with no interval change (thus excluding acute infection) and in two cases booking serology was tested and CMV IgG was positive suggesting the women were immune and the CMV IgM was a false positive in pregnancy.

Seven (1.4%) of the 496 (93.4%) women tested for Hepatitis B infection had Hepatitis B surface antigen detected of whom six had known diagnoses, including two in whom the diagnosis had been made at booking in the same pregnancy. The status of one woman who tested positive for Hepatitis B infection could not be ascertained as she had a single attendance at one hospital site whilst visiting London and had booked her pregnancy elsewhere.

Of the 421 (79.3%) women tested for Hepatitis C infection, three (0.7%) were positive, in all of whom the diagnosis had been made prior to the pregnancy. Of the 387 (72.9%) women tested for Hepatitis A infection, there were no positive IgM results.

Autoimmune testing

Autoantibody testing for smooth muscle antibodies

404 (76.1%) women were tested for smooth muscle antibodies of whom five (1.2%) were positive. None of the women with positive smooth muscle antibodies tested positive for any of the viral infections, anti-mitochondrial antibodies, or liver/kidney microsomal antibodies. ICP only was the recorded diagnosis in all cases on review of the electronic health records and clinic letters and none were referred to gastroenterology or hepatology services. Further details are given in Supplementary Text 1.

Autoantibody testing for antinuclear antibodies

Hep-2 antinuclear antibody (ANA) testing was performed as part of the ICP investigations at one hospital site only. Of the 227 women tested for Hep-2 ANA, 31 (13.7%) had antibodies detected, with only two at dilutions of 1:640 or 1:1280. Most women had known pre-existing diagnoses, including autoimmune diseases. On review of electronic health records and clinic letters, no new diagnoses other than ICP were made as a result of testing. Further details are given in Supplementary Text 2.

Autoantibody testing for anti-mitochondrial antibodies and liver kidney microsomal antibodies

403 (75.9%) women were tested for anti-mitochondrial antibodies, of whom five (1.2%) were positive. Three women were referred to gastroenterology/hepatology services and discharged after further investigations. In the remaining cases no referrals to specialist services were made and no new clinical diagnoses other than ICP were evident on electronic health records and clinic letters. Further details are given in Supplementary Text 3. Of the 406 women tested for liver kidney microsomal antibody there were no positive results.

Ultrasound testing for comorbidities and additional diagnoses

In total 327 (61.6%) women had a liver and gallbladder ultrasound as part of ICP investigations. There were high rates of co-incidental findings on imaging, shown in supplementary Table 1. Eighty-five women (26.2%) had gallbladder abnormalities detected including 60 women (18.5%) with gallstones and/or biliary sludge, 12 (3.7%) with a polyp, 10 (3.1%) with previous cholecystectomy, and three other minor abnormalities (debris noted in gallbladder, gallbladder wall thickened, mild gallbladder oedema).

Coagulation testing

348 women (65.5%) had coagulation tests performed. Three had a prolongation of prothrombin time; one also had severe pre-eclampsia and HELLP [haemolysis, elevated liver enzymes, low platelets] syndrome necessitating delivery at 30 weeks' gestation, and two had acute fatty liver of pregnancy.

Diagnoses in women presenting with early onset rise in bile acid concentrations

There were 23 women (4.3%) who had a peak bile acid concentration of ≥40 µmol/L at less than 32 weeks' gestation, ten (43.5%) of whom had a peak bile acid concentration of ≥100 µmol/L during pregnancy. In 12/23 cases (52.2%) ICP was the only liver diagnosis during pregnancy with no additional comorbidities of note. In the remaining cases pre-existing comorbidities, alternative explanations for the abnormal liver function tests and/ or additional pregnancy complications were present. Full details are in Supplementary Text 4 and Supplementary Table 2.

Characteristics of testing

In total 250/531 (47.1%) of women with raised bile acids during pregnancy had complete virology, autoimmune and ultrasound tests. Only 24 (4.5%) women had no additional tests. There were no significant differences in the proportion of women with peak bile acid concentration of 11-39 µmol/L, 40-99 µmol/L or ≥100 µmol/L or more that had full virology testing (p=0.244, 62.8%, 70.0%, 70.4% tested, respectively), or autoimmune testing (p=0.514, 74.4%, 78.5%, 79.6% tested respectively). However women with higher peak bile acids were more likely to have a liver ultrasound (p=0.015, 57.3%, 66.2%, 75.9% respectively).

Cost of additional investigations in ICP

The cost for a full set of ICP blood investigations (virology and autoimmune) for a single woman, estimated utilising Viapath Laboratory Services costs, was £130.67; the cost of an abdominal ultrasound within the NHS is estimated to be £150; coagulation screen costs £3.50. Therefore, we estimate around £35,000 per year was spent investigating women across the two hospitals. If all women had received all tests as recommended by the RCOG Green-top guidelines this would equate to £75,000 per year across the two sites.

Discussion

Principal findings

In our study cohort of pregnant women with raised serum bile acid concentrations of >10 µmol/L, there were no new diagnoses that resulted in ongoing specialist management detected from additional investigations routinely performed in clinical practice. Approximately one in five women who had bile acids tested in pregnancy had elevated serum bile acid concentrations of >10 µmol/L. Of the women with raised bile acid concentrations, approximately half of women had a full set of virology, autoimmune and ultrasound testing investigations, as currently recommended by national guidelines. Our data show that amongst women tested, over 95% were already immune to EBV. The cost of performing additional routine testing for ICP is substantial, for minimal, if any, new clinical information. There were no antenatal characteristics relating to the women, or the initial bile acid concentration, that identified a sub-group of women who might benefit from additional testing.

Strengths and weaknesses

The strength of our study is that it is a two-centre study in a large city with a multi-ethnic population addressing a real-world, clinical conundrum. However, as we selected women with raised bile acids in pregnancy rather than a clinically defined population of women with suspected ICP it is possible that our study included women with alternative diagnoses, increasing our denominator and so diluting our reported rates of investigation. Bile acids can be raised in several hepatobiliary conditions including hepatitis, obstructive jaundice and cirrhosis[7] as well as in pre-eclampsia, but bile acids are not usually tested in these conditions during or outside of pregnancy. In addition, the electronic health record review

was limited to the hospital at which the initial bile acid test was performed and where the care during pregnancy and the postpartum period was provided, so it is possible that some women with hepatic diseases, e.g. autoimmune hepatitis, were not ascertained, if electronic records did not adequately capture other specialty involvement subsequent to the pregnancy. However, as pregnancy outcome data were available for all but 25 (4.3%) women, it appears that the majority of women received antenatal care and delivered within the two hospital Trusts.

Comparison with other studies

In a UK series of 70 patients with ICP, ultrasound scans revealed gallstones in 10% of women, and two (2.9%) cases of Hepatitis C were detected as a result of virology testing.[8] In a French series reporting clinical characteristics of 50 women with ICP, virology testing did not reveal any concomitant liver disease and ultrasound examination was normal in all women.[9] In a published Scandinavian cohort of 91 women negative virology testing and normal liver ultrasound defined the ICP cohort and the number of women excluded on the basis of positive virology results were not reported.[10] One case in our study had genetic variation in ABCB4 and ABCB11, consistent with the findings of a UK study in which pathogenic mutations in these biliary transporters were identified in approximately 20% cases.[11] None of these studies specifically investigated the clinical utility of additional investigations in ICP. Recommendations in the RCOG guidelines for management of ICP were based on expert opinion alone.

Implications for clinicians and policymakers

Our findings suggest that the currently recommended routine additional investigations have no diagnostic yield and that a more targeted testing approach should be advocated. This is especially pertinent in an era in which healthcare efficiency is increasingly relevant, and the Academy of Medical Royal Colleges is encouraging avoidance of unnecessary testing and procedures through the Choosing Wisely programme (see: http://www.choosingwisely.co.uk/about-choosing-wisely-uk/). This extends to autoantibody testing, where a focus on not testing persons with a low pre-test probability of disease has been proposed.[12] Reducing unselected testing would save money and allow resources to be

focussed on women with atypical disease, clinical uncertainty, women with additional comorbidities or if postpartum resolution does not occur.

Conclusion

Our data suggest little diagnostic value of routine additional testing investigations in ICP in women presenting with typical features. A more targeted and individualised approach could be used, based on factors such as atypical clinical symptoms, presence of comorbidities, severity of ICP, gestational age at presentation or failure of liver function tests to normalise postpartum.

Declarations

Conflicting interests: The authors declare that there is no conflict of interest.

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Guarantor: LCC is the guarantor of this article.

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Figure 1. Flow diagram illustrating selection of women with raised bile acid concentrations in pregnancy. BA: bile acid.

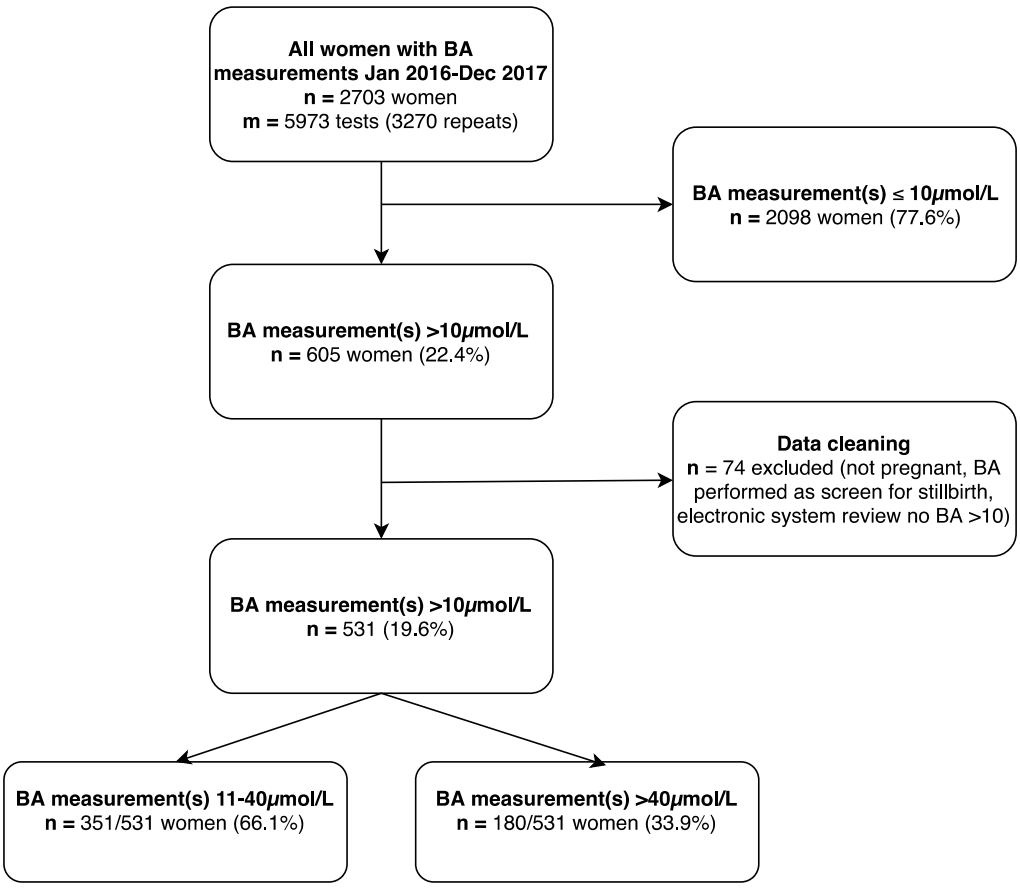

Table 1. Maternal demographics of women with bile acid concentration > 10 µmol/L during
IQR: interquartile range. BMI: Body mass index.

	Site 1 n = 317	**Site 2 n = 214**	**All women n = 531**
Age (median [IQR])	34 [30-37]	31 [27-35]	33 [29-36]
Age category (n(%))			
<20	1 (0.3)	5 (2.3)	6 (1.1)
20-29	65 (20.5)	69 (32.2)	134 (25.2)
30-39	184 (58.0)	123 (57.5)	307 (57.8)
>=40	47 (14.8)	17 (7.9)	64 (12.1)
Not recorded	20 (6.3)	0 (0.0)	20 (3.7)
Ethnic Origin (n (%))			
White	124 (39.1)	75 (35.0)	199 (37.5)
Asian	38 (12.0)	109 (50.9)	147 (27.7)
Black	40 (12.6)	19 (8.9)	59 (11.0)
Other	7 (2.2)	6 (2.8)	13 (2.4)
Mixed	9 (2.8)	2 (0.9)	11 (2.1)
Not recorded or declined	99 (31.2)	3 (1.4)	102 (19.2)
BMI (kg/m^2) (median [IQR])	23.6 [20.8-27.2]	25.0 [23.0-28.0]	24.0 [21.9-27.7]
BMI category (kg/m^2) (n (%))			
<20	37 (11.7)	13 (6.1)	50 (9.4)
20-24.9	131 (41.3)	84 (39.3)	215 (40.5)
25-29.9	65 (20.5)	73 (34.1)	138 (26.0)
30-34.9	17 (5.4)	27 (12.6)	44 (8.3)
>=35	15 (4.7)	15 (7.0)	30 (5.6)
Not recorded	52 (16.3)	2 (0.9)	54(10.1)
Parity (n (%))			
0	176 (55.5)	112 (52.3)	288 (54.2)
1	77 (24.3)	75 (35.0)	152 (28.6)
2	27 (8.5)	15 (7.0)	42 (7.9)
>2	18 (5.6)	12 (5.6)	30 (5.6)
Not recorded	19 (6.0)	0 (0.0)	19 (3.6)

Table 2. Delivery and perinatal outcomes in women with raised bile acid concentration in all women and grouped by peak bile acid concentration. Results displayed as median [interquartile range] or number (%).

		Peak bile acid concentration (µmol/L)			
Delivery outcomes	**All women n = 531**	**11-39 n = 347**	**40-99 n = 130**	**100 + n = 54**	**p-value***
Gestational age at delivery, weeks	37.9 [37.1-39.0]	38.1 [37.3-39.3]	37.4 [36.5-38.1]	37.1 [37.0-37.9]	**<0.001**
Onset					
Spontaneous labour	114 (21.5)	79 (22.8)	23 (17.7)	12 (22.2)	0.332
Induction of labour	265 (49.9)	173 (49.9)	71 (54.6)	21 (38.9)	0.540
Elective Caesarean section	103 (19.4)	69 (19.9)	26 (20.0)	8 (14.8)	0.955
Missing data	49 (9.2)	26 (7.5)	10 (7.7)	13 (24.1)	
Delivery					
<37 weeks	101 (19.0)	52(15.0)	38 (29.2)	11 (20.4)	**0.002**
- Spontaneous onset	26 (4.9)	10 (2.9)	12 (9.2)	4 (7.4)	
- Induction of labour	26 (4.9)	15 (4.3)	9 (6.9)	2 (3.7)	
- Elective caesarean section	34 (6.4)	21 (6.1)	11 (8.5)	2 (3.7)	
- Missing	15 (2.8)	6 (1.7)	6 (4.6)	3 (5.5)	
<34 weeks	21 (4.0)	12 (3.5)	7 (5.4)	2 (3.7)	0.631
- Spontaneous onset	7 (1.3)	3 (0.9)	3 (2.3)	1 (1.9)	
- Induction of labour	3 (0.6)	2 (0.6)	0 (0.0)	1 (1.9)	
- Elective caesarean section	7 (1.3)	5 (1.4)	2 (1.5)	0 (0.0)	
- Missing	4 (0.8)	2 (0.6)	2 (1.5)	0 (0.0)	
Missing data	22 (4.1)	11 (3.2)	4 (3.1)	7 (13.0)	
Number of babies					
1	463 (87.2)	308(88.8)	111 (85.4)	44 (81.5)	
2	47 (8.9)	29 (8.4)	15 (11.5)	3 (5.6)	0.431
Missing data	21 (4.0)	10 (2.9)	4 (3.1)	7 (13.0)	
Perinatal outcomes (n of babies)	**n = 578**	**n = 376**	**n = 145**	**n = 57**	
Mode of delivery					
Spontaneous vaginal delivery	259 (44.8)	176 (46.8)	60 (41.4)	23 (40.4)	0.454
Emergency Caesarean Section	124 (21.5)	74 (19.7)	34 (23.4)	16 (28.1)	0.163
Elective Caesarean Section	104 (18.0)	71 (18.9)	26 (17.9)	7 (12.3)	0.626
Assisted vaginal delivery	65 (11.2)	40 (10.6)	21 (14.5)	4 (7.0)	0.339
Missing data	26 (4.5)	15 (4.0)	4 (2.8)	7 (12.3)	
Birth outcome					
Livebirth	549 (95.0)	361 (96.0)	140 (96.6)	48 (84.2)	**0.001**
Intrauterine death	2 (0.3)	0 (0.0)	0 (0.0)	2 (3.5)	**<0.001**
Neonatal death	1 (0.2)	0 (0.0)	1 (0.7)	0 (0.0)	0.233
Missing data	26 (4.5)	15 (4.0)	4 (2.8)	7 (12.3)	
Birthweight, g	3096 (2688-3450)	3170 (2770-3500)	2920 (2530- 3310)	3014 (2508-3120)	**<0.001**

Birthweight <2500 g	91 (15.7)	46 (12.2)	34 (23.4)	11(19.3)	**0.005**
Male	298 (51.6)	198 (52.7)	73 (50.3)	27 (47.4)	0.825
Female	254 (43.9)	163 (43.4)	68 (47.9)	23 (40.4)	
Missing data	26 (4.5)	15 (4.0)	4 (2.8)	7 (12.3)	
Neonatal unit admission	77 (13.3)	43 (11.4)	29 (20.0)	5 (8.8)	**0.030**
Missing data	26 (4.5)	15 (4.0)	4 (2.8)	7 (12.3)	

*p-values denoting results of tests for difference between peak bile acid concentration groups (11-39, 40-99 and ≥100µmol/L).

Table 3. First raised bile acid concentration (> 10 µmol/L), liver function test results and gestational age at time of first raised bile acid result in all women and grouped by subsequent peak bile acid concentration.

		Peak bile acid concentration (µmol/L)			
Liver function test results, median [IQR]	**All women n = 531**	**11-39 n = 347**	**40-99 n = 130**	**100 + n = 54**	**p-value***
First bile acid > 10 µmol/L	18 [13-32]	15 [12-20]	41 [25-55]	59 [25-127]	**<0.001**
Gestation (weeks)	35.1 [31.8-37.0]	35.7 [31.4-37.3]	34.6 [32.4-36.6]	33.4 [30.3-36.3]	0.188
Bilirubin (µmol/L)	7 [5-10]	6 [4-9]	7 [5-11]	9 [6-15]	**<0.001**
Alanine transaminase (IU/L)	40 [20-106]	31 [17-76]	72 [36-145]	78 [40-189]	**<0.001**
Gamma-glutamyl transferase IU/L)	21 [14-35]	20 [12-31]	25 [16-44]	26 [14-38]	**0.021**

*Comparing biochemistry results between peak bile acid concentration groups 11-39, 40-99 and 100+ µmol/L.

Table 4. Summary of screening investigations (virology, autoimmune and ultrasound) in women with raised bile acid concentration during pregnancy.

	Number (%) of women tested	Number (%) positive result	Number (%) by peak BA concentration (µmol/L)			Number (%) known diagnosis	Number (%) new diagnosis
Virology			11-39	40-99	100+		
EBV serology All women (n=531)	380 (71.6)	1/380 (0.3)	0/238 (0.0)	1/99 (1.0)	0/43 (0.0)	0/1 (0.0)	0/188 (0.0)
CMV serology All women (n=531)	395 (74.4)	0/395 (0.0)	-	-	-	-	-
Hepatitis C IgG All women (n=531)	421 (79.3)	3/421 (0.7)	1/266 (0.4)	1/109 (0.9)	1/46 (2.2)	3/3 (100)	0/421 (0.0)
Hepatitis B sAg All women (n=531)	496 (93.4)	7/496 (1.4)	4/327 (1.2)	2/119 (1.7)	1/50 (2.0)	6/7 (85.7) 1/7*(14.3)	0/496 (0.0)
Hepatitis A IgM All women (n=531)	387 (72.9)	0/387 (0.0)	-	-	-	-	-
Autoimmune							
Smooth muscle Ab All women (n=531)	404 (76.1)	5/404 (1.2)	2/259 (0.8)	3/102 (2.9)	0/43 (0.0)	0/5 (0.0)	0/406 (0.0)
Hep2 Antinuclear Ab Site 1 (n=317)	227 (71.6)	1/227 (0.4)	1/133 (0.8)	0/65 (0.0)	0/29 (0.0)	1/1† (100)	0/227 (0.0)
Mitochondrial Ab All women (n=531)	403 (75.9)	5/403 (1.2)	3/258 (1.1)	0/102 (0.0)	2/43 (4.7)	1/5‡ (25.0)	0/405 (0.0)
Liver/kidney microsomal Ab All women (n=531)	406 (76.5)	0/406 (0.0)	-	-	-	-	-
Imaging							
Liver ultrasound All women (n=531)	326 (61.4)	38/326 (11.7)	24/199 (12.1)	7/86 (8.1)	7/41 (17.1)	-	0/327 (0.0)
Gallbladder ultrasound All women (n=531)	323 (60.8)	85/323 (26.3)	45/197 (22.8)	26/86 (30.2)	14/40 (35.0)	-	0/324 (0.0)

*Not booked at GSTT, single attendance. EBV: Epstein Barr Virus. CMV: Cytomegalovirus. sAg: surface antigen. Ab: antibody.

†Known Ro and anti-PL-12 positive anti-synthetase syndrome positive interstitial lung disease prior to pregnancy.

‡Known rheumatoid arthritis prior to pregnancy.

Supplementary Tables

Supplementary Table 1. Liver and gallbladder findings in women with raised bile acids.

	All women (n=327)
Gallbladder abnormalities	
Gallstones and/or biliary sludge	60 (18.5)
Polyp	12 (3.7)
Cholecystectomy	10 (3.1)
Other*	3 (0.9)
Total	85 (26.2)
Liver abnormalities	
Fatty infiltration	20 (6.1)
Haemangioma†	10 (3.1)
Cyst	3 (0.9)
Other‡	3 (0.9)
Heterogenous echotexture§	2 (0.6)
Total	38 (11.6)

*1 – debris noted in gallbladder, 2 - gallbladder wall thickened, 3 – mild gallbladder oedema.

†In one case haemangioma was picked up in a woman with known Hepatitis C.

‡1 - Small calcific focus, 2 - prior surgical resection, 3 - known focal nodular hyperplasia who had further ultrasound in pregnancy to determine if there was any enlargement.

§In one case heterogenous echotexture was noted in a woman known Hepatitis B who had regular liver ultrasound scans.

Supplementary Table 2. Rates of positive test results comparing between women with severe early-onset disease and those without.

	Severe early-onset disease (peak BA ≥40 µmol/L at <32/40 weeks gestation) n=23	**Non severe early-onset disease n=508**
Hep C IgG	1/21 (4.8)	2/400 (0.5)
Hep B sAg	0/22 (0.0)	7/474 (1.5)
Hep A IgM	0/22 (0.0)	0/368 (0.0)
Smooth Muscle Ab	1/17 (5.9)	4/387 (1.0)
Mitochondrial Ab	1/17 (5.9)	4/386 (1.0)
Liver/kidney microsomal Ab	0/23 (0.0)	0/387 (0.0)
Liver pathology	2/20 (10.0)	36/306 (11.8)
Gallbladder pathology	7/20 (35.0)	78/303 (25.7)

Supplementary Text

Supplementary Text 1

Autoantibody testing for smooth muscle antibodies

404 (76.1%) women were tested for smooth muscle antibodies of whom five (1.2%) were positive: three at 1:80 dilution and two at 1:40. One woman also had Hep-2 ANA antibodies detected at 1:80 dilution. Median liver function test results in these women at time of first raised bile acid were: bilirubin 7 (IQR 4-7) µmol/L, alanine transaminase 104 (IQR 21-186) IU/L, and gamma-glutamyl transferase 31 (15-38) IU/L. Liver function tests (other than bile acid concentrations) were normal during pregnancy in one woman, resolved postpartum in two women, were not checked post-delivery in one woman and remained elevated (62IU/L) at 2 months postpartum in one woman.

Supplementary Text 2

Autoantibody testing for antinuclear antibodies

Hep-2 antinuclear antibody (ANA) testing was performed as part of the ICP investigations at one hospital site only. Of the 227 women tested for Hep-2 ANA, 31 (13.7%) had antibodies detected; 14 at 1:40 dilution, 12 at 1:80 dilution, 3 at 1:160 dilution, 1 at 1:640 dilution and 1 at 1:1280 dilution. Several women had pre-existing autoimmune disease diagnoses including the woman with an ANA titre of 1:1280 who had a diagnosis of fibrotic interstitial lung disease, anti-synthetase syndrome and was also known to be anti-Ro and anti-PL-12 antibody positive. She was not referred for hepatology specialist review as her liver function tests remained in the normal range throughout pregnancy and her raised antibodies were considered to be related to her autoimmune lung disease. Other pre-existing diagnoses in this group of women were sickle cell disease with antiphospholipid syndrome (1:80 dilution), Grave's disease (1:80 dilution) and ulcerative colitis (1:40). On review of electronic health records and clinic letters, no new diagnoses other than ICP were made as a result of testing.

Supplementary Text 3

Autoantibody testing for anti-mitochondrial antibodies

403 (75.9%) women were tested for anti-mitochondrial antibodies, of whom five (1.2%) were positive; three at dilutions of 1:40; one woman tested negative on immunoblot

testing, and one woman tested positive on immunoblot testing. Median liver function test results at time of first raised bile acid were: bilirubin 7 (IQR 7-11) µmol/L, alanine transaminase 47 (IQR 17-330) IU/L, and gamma-glutamyl transferase 17 (14-23) IU/L, Liver function tests other than bile acids were normal throughout pregnancy in one woman. In the four cases that were elevated during pregnancy, three normalised postpartum, and in one case the woman was followed up in primary care (results not available). Three women were referred to gastroenterology services: one was seen by a hepatologist and had liver and biliary magnetic resonance imaging which was normal and no further follow up was arranged, one was seen by a gastroenterologist and discharged, and in the final case the woman that tested positive on immunoblot testing was seen by a hepatologist, had a normal fibroscan and was discharged. In the remaining cases no referrals to specialist services were made and no new clinical diagnoses other than ICP were evident on electronic health records and clinic letters.

Supplementary Text 4

Diagnoses in women presenting with early onset rise in bile acid concentrations

There were 23 women (4.3%) who had a peak bile acid concentration of ≥40 µmol/L at less than 32 weeks' gestation, ten (43.5%) of whom had a peak bile acid concentration of ≥100 µmol/L during pregnancy. In 12/23 cases (52.2%) ICP was the only liver diagnosis during pregnancy with no additional comorbidities of note. In the remaining cases pre-existing comorbidities, alternative explanations for the abnormal liver function tests and/ or additional pregnancy complications were present. In four cases (18.2%), abnormal liver function tests prior to pregnancy, early in pregnancy or postpartum were noted. In one case a woman booked with abnormal liver function but was lost to follow up after delivery, and in one case a woman had a history of abnormal ALT and bilirubin prior to pregnancy with multiple episodes of suspected biliary colic postnatally suggesting gallstone disease rather than ICP. Two women had persistently abnormal LFTs postpartum; one woman was investigated and found to be heterozygous for the ABCB4 genetic variant (Arg1046Ter) and homozygous for the ABCB11 Val444Ala genetic variant and in the other, the woman had not attended for further follow-up. Two women (9.0%) had known inflammatory bowel disease as well as ICP (one case of known Crohn's disease and Primary Sclerosing Cholangitis, one case of known Ulcerative Colitis and Hepatitis C positive), one woman had known

seropositive rheumatoid arthritis (and tested positive for anti-mitochondrial antibodies), in one woman cholestasis could have been triggered by an antibiotic reaction following several courses of antibiotics for pyelonephritis in pregnancy, one woman developed pre-eclampsia and HELLP syndrome, one woman was treated for tuberculosis of the spine during pregnancy but subsequently was diagnosed postpartum with metastatic cancer of unknown primary, and one woman was not diagnosed with ICP but had an episode of gallstone pancreatitis during pregnancy.

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Ethical approval: The study was registered as an audit at both local hospital sites and approved by the Trust Audit Committee. Audit Numbers: GSTT1000 (Site 1), WCSLA 043 (Site 2).

Trial registration: Not applicable because study was a retrospective audit.

Contributorship: LCC and JG designed the study. FCR, MM, RH, CLK, GA , OB and JW performed data collection. FCR and RH performed data analysis. FCR, CW, LCC and JG interpreted the results. The first manuscript draft was written by FCR and re-drafted and approved by all other authors.

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